induces membrane disruption to a similar extent as the full-length peptide. However, unlike the full-length IAPP peptide, IAPP1-19 is conformationally stable in a helical conformation when bound to the membrane. In vivo and in vitro measurements of membrane disruption indicate the rat version of IAPP1-19, despite differing from hIAPP1-19 by only by the single substitution of Arg18 for His18, is significantly less toxic than hIAPP1-19, in agreement with the low toxicity of the full-length rat IAPP peptide. To investigate the origin of this difference at the atomic level, we have solved the structures of the human and rat IAPP1-19 peptides in DPC micelles, as well as the completely non-toxic full-length rat and toxic full-length human peptide. While the structures of rat and human IAPP1-19 are similar, the charge at residue 18 plays a key role in controlling the toxicity of the peptide. At pH 7.3, the more toxic hIAPP1-19 peptide is buried deeper within the micelle, while both the less toxic rIAPP1-19 peptide and non-toxic full-length rIAPP peptide are located at the surface of the micelle. Deprotonating H18 in hIAPP1-19 moves the peptide to the surface of the micelle. This change in orientation is accompanied by a corresponding change in toxicity. At pH 6.0, the membrane disruption induced by hIAPP1-19 is significantly decreased and resembles that of the less toxic rIAPP1-19 peptide.

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Amyloidogenic Propensity of ProIAPP and IAPP in the Presence of Negatively Charged Lipid Bilayers

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The islet amyloid polypeptide (IAPP) is synthesized in the β-cells of the pancreas from its precursor, the proislet amyloid polypeptide (ProIAPP). ProIAPP is co-processed in the secretory granules, and then co-secreted to the extracellular matrix together with insulin. As indicated earlier, partially processed N-terminal ProIAPP is able to interact with negatively charged moieties, like heparan sulfate, which may lead to islet amyloid plaque formation. Amyloid plaques have been found both extracellularly and intracellularly in type II diabetic patients. Here, we studied the amyloidogenic propensity of native ProIAPP and compared it with that of IAPP in the absence and presence of negatively charged membranes. Our CD studies show that the secondary structure content of ProIAPP and IAPP is predominantly unordered with small amounts of ordered secondary structure elements as confirmed by ATR-FTIR spectroscopy. However, in the presence of anionic membranes, ProIAPP forms predominantly α-helices and loops that subsequently transform to intermolecular β-sheet structures. For comparison, IAPP forms intermolecular β-sheets largely via unordered and loop structures. The ATR-FTIR and fluorescence spectroscopy studies performed also reveal that ProIAPP has a higher amyloidogenic propensity in the presence of negatively charged membranes, but is still less amyloidogenic than IAPP. AFM studies have also been carried out which show that ProIAPP, at variance to IAPP, does not form long fibrils, but rather protofilaments or short fibrils, only. Hence, both, the presence of a small amount of unprocessed ProIAPP in β-cell secretion, or the interaction with negatively charged surfaces, like negatively charged lipid bilayers, may initiate islet amyloid plaque formation.

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Structural Studies Of Islet Amyloid Polypeptide In The Presence Of Insulin And Lipid Membranes

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Previous work by others has shown that insulin inhibits the formation of fibrils from islet amyloid polypeptide. The mechanism for the inhibition is not known. To address this issue, we are studying IAPP in the presence of lipid membranes, with or without insulin. Circular dichroism spectra of IAPP alone in phospholipid membranes show that it undergoes a structural rearrangement from α-helix to a β-sheet conformation. In the presence of insulin, this transition is not observed, that is, IAPP remained a-helical. To explain this, we are using other biophysical methods including solution-state NMR, electron microscopy, SDS-PAGE and limited proteolysis monitored by mass spectrometry. Results from these studies will be presented and discussed in this poster.

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Amyloid-like Misfolding Of Peptides By Membrane Mimicking Environments

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Sodium dodecyl sulfate has been proven as an amyloid-like misfolding agent [1-3]. Our study comprises the biophysical characterization of human peptides in the presence of submicellar and micellar concentrations of SDS. The prodynorphin derived peptides (Big dynorphin, dynorphin A and dynorphin B) [4], the amyloid β peptide [5], and the proinsulin derived C-peptide are our subject of study. As determined by CD and FTIR spectroscopy, the peptide structural transitions involve different secondary structures, such as random coil, β-sheet and α-helix. By means of NMR, dynamic light scattering, native-PAGE or ThT fluorescence, we have shown that all the peptides transit through a high molecular weight aggregated state at submicellar detergent concentrations. Finally, studies with model membranes with different charge composition have been carried out to relate the structural characterization of these peptides to their possible role in the cell and their action mechanisms in pathology.

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Modeling amyloid toxic ion channels

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Non-amyloidogenic beta peptides, p3 ($A\beta_{17-42}$) and $A\beta_{11-42}$, resulting from α-secretase and BACE cleavage are often found in amyloid plaques. However, the biophysical properties and functional role of these non-amyloidogenic peptides are not understood. We present molecular dynamics (MD) simulations of channels consisting of the U-shaped *beta-strand-turn-beta-strand* peptides using available NMR-based coordinates of p3 and $A\beta_{9-42}$. Our results show that non-amyloidogenic p3 and $A\beta_{9-42}$ peptides form ion channel-like structures with loosely attached subunits. These channels are dynamic and are made of small peptide oligomers. The channels can conduct calcium and obtain shapes and dimensions consistent with Atomic Force Microscopy (AFM) images. All channels break into mobile subunits suggesting that membranes do not support intact β-sheet channels. We shall further present results of modeling both PG-1 and k3-β_2m channels, presenting a consistent general picture of toxic beta-sheet based channels. Funded in part by DHHS #N01-CO-12400.

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Probing Tau-Vesicle Interactions

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Tau is the major protein component of the neurofibrillary tangles that characterize a group of neurodegenerative diseases, including Alzheimers Disease. The transformation of tau from its native state where it functions as a microtubule-associated protein (MAP), to its pathological state as is found in patients suffering from any of the various associated diseases, is not well understood. Studies have shown that tau aggregation can be induced by anionic lipid vesicles, and that detergent micelles can induce folding of the microtubule binding domain of tau. Here we use fluorescence correlation spectroscopy (FCS) to monitor the interaction of tau with synthetic lipid vesicles, in order to investigate vesicle binding and aggregation. Studying several isoforms of tau, we find that solution pH plays a strong role in such interactions.

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An α-Helical Conformation of the SEVI Peptide, a Dramatic Enhancer of HIV Infectivity, Promotes Lipid Aggregation and Fusion

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A peptide ubiquitous in human seminal fluid has been recently described that dramatically enhances the infectivity of the HIV virus (3-5 orders of magnitude by some measures). Previous studies have shown that this peptide, a fragment of human Prostatic Acid Phosphatase ($PAP_{248-286}$) referred to as SEVI (Semen-derived Enhancer of Viral Infection), is amyloidogenic and the enhancement of viral infectivity is dependent on the aggregation state of the peptide. To complement these previous in vivo studies we have performed in vitro assays to investigate the physical mechanisms by which the $PAP_{248-286}$ promotes the interaction with lipid bilayers. Our results indicate a strong interaction of freshly dissolved $PAP_{248-286}$ with lipid bilayers but a weaker interaction with the amyloid form of $PAP_{248-286}$, as measured by the tendency of freshly dissolved $PAP_{248-286}$ to induce aggregation of lipid vesicles and membrane fusion. The

amyloid form of $PAP_{248-286}$ had little effect on either vesicle aggregation or fusion. To further investigate this effect we have solved the structure of $PAP_{248-286}$ in SDS micelles. A largely α-helical conformation of $PAP_{248-286}$, lying parallel to the membrane surface, is implicated in promoting bridging interactions between membranes by the screening of the electrostatic repulsion that occurs when two membranes are brought into close contact. This suggests non-specific binding of small oligomeric forms of SEVI in an α-helical conformation to lipid membranes may be an additional mechanism by which SEVI enhances the infectivity of the HIV virus.

Lipids and Signaling on Membrane Surface

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The Preferential Reconstitution Of Ampa Receptor Proteins Into Model Lipid Domains With Cholesterol Studied By Atomic Force Microscopy - an Imaging And Force Spectroscopy Study

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In our research we have conducted an atomic force microscopy (AFM) study of trafficking-like behaviour of neural receptor proteins into lipid raft-like domains. In our initial research we formed artificial rafts by varying a mixture of four phospholipids found in the synapse in order to mimic a synaptic membrane. The most commonly occurring receptor protein in the central nervous system, the AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), was then reconstituted into these mixtures. The results show a preferential reconstitution of these membrane proteins into lipid rafts of a certain height. AMPA receptors are implicated in long term potentiation, a process thought to underlie learning and memory, with up-regulation of AMPAR numbers in the post-synaptic membrane possibly being a key component of this process.

In order to come closer to the mixtures naturally occurring in the synapse we furthered these studies to incorporate cholesterol. The results were a preferential reconstitution of AMPAR proteins but this time into the low domain when cholesterol is present. These surprising results were better understood when we treated this system as a ternary mixture with gel phase lipids, liquid phase lipids and cholesterol acting as an impurity. We studied the phases in terms of the domain heights as well as their mechanical properties. When cholesterol was present, the protein-deficient high domains were stiffer and more viscous.

The lateral extent of the lipid domains is typically ~100nm, so they have structural similarities with the lipid rafts observed to occur in synaptic membranes, albeit with much simpler composition. Dynamic AFM measurements reveal information about the mobility of receptors within and between domains which may shed light on this process.

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Piezoelectricity of phospholipids: Are cell membranes also piezoelectric?

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Recently it was found[i] that mechanical deformation of films of L-α-phosphatidylcholine in the L_a phase induces an electric polarization. It was suggested that this effect is due to the chiral smectic A (SmA*) type liquid crystal structure of the bilayers, which under molecular tilt becomes a ferroelectric (SmC*) phase, where the electric polarization is normal to the tilt plane. However no control measurement on the racemic material has been presented to prove this suggestion. Here we demonstrate that indeed the chirality of phospholipids makes fluid lipid bilayers piezoelectric. By periodically shearing and compressing nonaqueous lamellar phases of synthetic right enantiomer 2,3-Dihexadecanoyl-sn-glycero-1-phosphocholine (D-DPPC) the synthetic left enantiomer 1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine (L-DPPC) lipids and their racemic mixture (DL-DPPC) ,we induced a tilt of the molecules with respect to the bilayer normal and produced electric current perpendicular to the tilt plane, with the chiral lipids only. Because most of the living cell membranes contain chiral lipids, we hypothesize that piezoelectricity may have a role in the function of cell membranes. For example, this coupling allows for a wide variety of sensory possibilities of cell membranes such as mechano-reception, magneto-sensitivity, and proton membrane transport. Preliminary results on electromechanical couplings in *Saccharomyces cerevisiae* (Baker's yeast) and their protoplasts will be also reported and discussed.

Endnotes
[i] A. Jákli, J. Harden, C. Notz and C. Bailey, *Liquid Crystals,* 35 (4), 395-400 (2008).

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Gastrin-Releasing Peptide Adopts An Orientation Parallel To The Membrane Plane As A Preferred Orientation In DMPC Bilayers: Multiple Molecular Dynamics Simulations

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Gastrin-releasing peptide (GRP) binds to GRP-receptor (GRPR), a member of GPCR family. GRP is one of the bombesin peptides and they are implicated in obesity and cancer. Understanding the mechanism of GRP-GRPR interactions at molecular level is extremely significant and requires the knowledge of the structure of peptide-receptor complex. Since the complex structure is not available, the structures of ligand and the free receptor could be used to model the complex. GRP is flexible in aqueous medium but it is likely to adopt a stable structure when it binds to membrane according to ''Membrane Compartments Theory'' [*Biopolymers* **37**, 5-16 (1995)].

The C-terminal decapeptide of GRP is biologically active and is modeled as a helix using a related peptide structure determined in SDS micelles [*FEBS Lett.* **460**, 263-269 (1999)]. Its amino acid sequence is GNHWAVGHLM. We carried out multiple independent simulations of GRP peptide in explicit DMPC bilayers which differed in the orientation of GRP inside the bilayers and force-field. At the end of 10 to 20 ns production runs, five out of six simulations resulted in the peptide orientation that is nearly parallel to the membrane plane. This indicates that this orientation is a preferred one and is independent of CHARMM or GROMOS force-fields. In the sixth simulation, the peptide was deeply inserted inside the bilayer. We analyzed the stability of helix, interaction of individual residues with different lipid components and water penetration in both layers. The helix structure is stable in majority of the simulations. Our results indicate that the residues Gly-7 and His-8 are important in maintaining the helical structure and orienting the peptide.

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Lipid Composition Modulates the Stability of DNA Acting as Model Membrane-bound Receptors

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Many important signaling processes occur in the interactions between lipid organelles: a multitude of ligands and receptors are localized to the surface of lipid structures and vary in many ways, including their length and the strength of their interactions. DNA strands with hydrophobic modifications anchor to the surface of lipid membranes. These membrane-anchored DNA diffuse within the lipid matrix and can bind specifically to their complement: minimal properties of real membrane receptors. The properties of these DNA ''receptors'' can be varied systematically to explore the physical advantages of variables such as receptor length, binding strength and repeated sequences in the binding domain.

We show that the binding equilibrium between DNA-functionalized vesicles is dependent upon lipid composition. We develop a model as a framework to understand this phenomenon by extension of the Bell model to the non-constant force-fields between lipid membranes. We find that the inter-membrane interactions can either suppress or favor receptor binding and discuss the possible implications for biological receptor-mediated signaling processes.

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Phosphatidylinositol-(4,5)-bisphosphate Acting As A Ligand Of PKCalpha Modulates The Membrane Localization Of This Enzyme In Living Cells

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Rapamycin-triggered heterodimerization strategy is becoming an excellent tool for rapidly modifying phosphatidylinositol(4,5)-bisphosphate [PtdIns(4,5)P2] levels at the plasma membrane and for studying their influence indifferent processes. In this work, we studied the effect of modulation of the PtdIns(4,5)P2 concentration on protein kinase C (PKC)α membrane localization in intact living cells. We showed that an increase in the PtdIns(4,5)P2 concentration enlarges the permanence of PKCα in the plasma membrane when PC12 cells are stimulated with ATP, independently of the diacylglycerol generated. The depletion of this phosphoinositide decreases both the percentage of protein able to translocate to the plasma membrane and its permanence there. Our results demonstrate that the polybasic cluster located in the C2 domain of PKCα is responsible for this phosphoinositide-protein interaction. Furthermore, the C2 domain acts as a dominant interfering module in the neural differentiation process of PC12 cells, a fact that was also supported by the inhibitory effect